MUTATION IN MAIZE

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Studies of controlling elements in maize were continued during the past year, with the purpose of learning more about the ways in which they control gene action and the types of action induced. Some of the results of these studies will be reviewed here. In addition, an investigation was begun of a particular alteration in structural organization of one chromosome of the maize complement, which has been found to induce still other changes, not only in chromosome organization but also in gene expression. A review of this case will be included.

Ac CONTROL OF MUTATION AT THE BRONZE LOCUS IN CHROMOSOME 9

The insertion of the controlling element *Ac* at the bronze locus in chromosome 9, and its effects on gene action at that locus, were mentioned in Year Book No. 54. Study of this case was continued in order to extend the evidence that mutation is related to removal of *Ac* from the locus or to other events induced by *Ac*. The phenotype appearing when *Ac* resides at or close to the bronze locus is similar to that produced by the standard recessive, *bz*. Mutations occur, and give rise either to a *Bz* type of expression of genic materials at the locus or to a stable recessive expression similar to that given by the standard recessive, *bz*. Mutations leading to a stable recessive expression occur more frequently than those producing a *Bz*-type expression, as is made apparent in the frequency of appearance of germinal mutations when plants that are homozygous for the bronze locus with *Ac* are used as females in crosses with plants that are homozygous for the standard *bz* allele, which is stable in the presence of *Ac*. Eleven such plants were used as female parents in this type of cross; and among the total of 2552 kernels that appeared on the ears they produced, 2398 exhibited spots of *Bz* in a recessive bronze background—the type of expression that appears when *Ac* is present at the bronze locus in the initial endosperm nucleus of the kernel. In addition, 28 kernels were totally *Bz* in phenotype, and 126 were totally bronze, with no spots of the *Bz* phenotype. Mutation to a stable *bz* expression is thus four and a half times more frequent than mutation to *Bz*. This same ratio for the two main types of germinal mutation also appears on ears of plants that are heterozygous for the bronze locus with *Ac*. Plants used in tests to identify these mutations were also made heterozygous for two additional genetic markers located to either side of the bronze locus. These plants were used as female parents in crosses with plants that were homozygous for the standard recessive alleles. Table 3 shows the types of kernels that appeared on the ears of the tested plants. A double crossover involving the regions *sh* to *bz* and *bz* to *wx* is rare. Therefore, the numbers of totally *Bz* and totally *bz* kernels shown in the *Sh Wx* class of section A of the table and in the *sh wx* class of section B represent, on the whole, the numbers of germinal mutations that occurred at the bronze locus with *Ac* inserted. It will be seen that the ratio of the two types of germinal mutation is the same as that produced by the homozygotes.

In order to determine the part played by *Ac* in the mutation process, plants were grown from some of the *Bz* kernels and also from some of the totally *bz* kernels in the *Sh Wx* noncrossover class of section A of table 3. All the plants that gave rise to these kernels had been tested for *Ac* number and location; and selections of kernels showing germinal mutations were made only from plants having one *Ac*, residing at the mutable bronze locus. Plants were grown from 16 kernels that exhibited the *Bz* phenotype. Each was tested for presence or absence of *Ac*, for its location if present, for transmission of the chromosome carrying the *Bz* mutation through pollen and egg, for crossover relations with the linked markers, for stability of the *Bz* mutation in the presence of *Ac*, and for viability of the homozygote. Of the sixteen *Bz* mutants, fourteen proved to be stable. In six of the fourteen plants that had such a stable *Bz* mutation, *Ac* was absent. In five plants, one *Ac* was present but it was no longer located close to *Bz:* in four cases it was not linked with markers carried in chromosome 9, and in one case it was located close to *Wx*. In the three remaining plants, one *Ac* was present and it was located close to but probably to the right of *Bz*. Thus, in eleven of these fourteen cases of mutation to a stable *Bz* allele, it could be shown that the mutation-producing event was associated with removal of *Ac* from the bronze locus. In five cases, it was transposed to a new location. It may well be that such transpositions of *Ac* were also responsible for the origin of the *Bz* mutant in the six cases in which *Ac* was absent in the plant derived from the *Bz* kernel. If the transposition had occurred in a premeiotic cell, segregation in the subsequent meiotic mitoses of the chromosome carrying *Ac* in its new location could have produced a gamete carrying the *Bz* mutation but lacking *Ac*.

The *Bz* mutation in two of the plants derived from the 16 selected kernels that showed a *Bz* phenotype proved to be unstable. In both plants, *Ac* was present and located close to the locus of *Bz*. It could be shown that the mutations which subsequently occurred were instigated by this *Ac* element. Some of them effected stability of *Bz* expression. Three cases of stable *Bz* expression were examined, and in all three the stability was found to be associated with removal of *Ac* from the vicinity of the *Bz* locus. Other mutations effected return to the unstable recessive, and in the eight cases that were examined *Ac* was present and was responsible for the instability. Still others gave rise to stable recessives, but none of these was examined for the presence or absence of *Ac*. Mutations of other kinds were also noted; but only one, a very rare type, will be mentioned here. This mutation caused a marked reduction in anthocyanin pigmentation. The event responsible for it was associated with removal of *Ac* from the *Bz* locus and its insertion elsewhere. It proved to be stable only in the absence of *Ac;* if *Ac* was present somewhere in the chromosome complement, mutations occurred to produce alleles having either a higher or a lower level of anthocyanin pro-

TABLE 3

PHENOTYPES OF KERNELS APPEARING ON EARS PRODUCED BY PLANTS HETEROZYGOUS FOR THE BRONZE LOCUS WITH *Ac*, AND FOR GENETIC MARKERS LOCATED TO EITHER SIDE OF THIS LOCUS, WHEN CROSSED BY PLANTS HOMOZYGOUS FOR THE RECESSIVE ALLELES

A. ♀ *Sh bz⁀Ac Wx/sh bz wx* × ♂ *sh bz wx/sh bz wx*

B. ♀ *sh bz⁀Ac wx/Sh bz Wx* × ♂ *sh bz wx/sh bz wx*

Phenotype of kernel with respect to alleles of *Sh* and *Wx*	A Phenotype of kernel with respect to bronze			B Phenotype of kernel with respect to bronze		
	Spots of *Bz* in bronze background	Totally *Bz*	Totally *bz*	Spots of *Bz* in bronze background	Totally *Bz*	Totally *bz*
Sh Wx	2457	41	158	1	4	927
sh wx	0	1	2596	881	12	56
Sh wx	641	3	87	35	0	230
sh Wx	49	0	671	202	0	58

duction. They represented a change from apparently direct control of mutation by *Ac* to the "*Ds-Ac*" type of control. Two other cases of change to this type of control of the mutation process were found, and these will be discussed shortly.

Twenty-four cases of what appeared to be mutation to a stable recessive (*bz*) expression were also examined. Three of them occurred early in plant development, in three different plants. Each was made evident by the appearance, on one ear of the plant, of a sector in which all the kernels were totally bronze in phenotype; that is, none of the kernels within the sector exhibited any *Bz* spots. Plants were grown from some of the *Sh* and *sh* kernels in each of these three sectors, and were tested for the presence or absence of *Ac*, and for its location if present. Plants derived from the *Sh* class of kernels were also tested for stability of the *bz* mutant in the presence of *Ac*, for transmission through pollen and egg of the chromosome carrying the mutant, and for viability of the mutant when homozygous. From these tests it was learned that in each of the three cases a mutation to stable *bz* expression, occurring in a somatic cell early in plant development, had led to the appearance of the sector. Chromosomes carrying the mutations were normally transmitted through pollen and egg, and the homozygotes were viable. The mutation process was associated in all three cases with removal of *Ac* from the bronze locus. None of twelve plants derived from 7 *Sh* and 5 *sh* kernels from one of these sectors had *Ac*, although it was known that *Ac* was present at the bronze locus in the *Sh*-carrying chromosome in other kernels on this ear. In the other two cases, it could be determined with certainty that the mutation to the stable recessive was associated with transposition of *Ac* to a new location. In twenty-one plants (twelve derived from *Sh* kernels and nine derived from *sh* kernels), one *Ac* was found to be present. In the remaining fourteen plants (eight derived from *Sh* kernels and six derived from *sh* kernels), no *Ac* was present. In those plants having *Ac* that were also heterozygous for markers carried in chromosome 9, no linkage of *Ac* with these markers was evident.

Early-occurring transposition of *Ac* is relatively rare, so that sectors of the type described above are not frequently observed. Usually, transposition of *Ac* occurs relatively late in the development of plant tissues, including the sporogenous tissue. Germinal mutations are recognized in single kernels distributed more or less randomly on an ear. Twenty-one of the twenty-four examined cases of mutation to stable *bz* were derived from kernels of this type, in the *Sh Wx* class, which exhibited no spots of *Bz* but appeared to be completely bronze in phenotype. Plants grown from them were tested for the presence or absence of *Ac*, for its location if present, for stability of the bronze expression in the presence of *Ac*, for transmission of the mutant through pollen and egg, and for viability of the mutant when homozygous. In all twenty-one cases, the mutation was normally transmitted through pollen and egg and the homozygotes were viable. In eight of the twenty-one plants, no *Ac* was present; and in all eight cases the *bz* expression proved to be stable when *Ac* was introduced into a nucleus carrying the *bz* mutant. In three other plants, one *Ac* was present but it was not linked with markers in chromosome 9; these *bz* mutants also were stable. In another three plants, one *Ac* was present, and was located in chromosome 9—close to *Wx* in one plant, close to *Sh* in another, and close to *bz* in the third. In these three cases also the *bz* mutant proved to be stable. In six other plants one *Ac* was present and was carried in chromosome 9, but its exact location within the chromosome was not determined. Recombination with *Wx* ranged from 20 to 35 per cent. It is suspected that *Ac* may have been located close to the *bz* locus in at least two of these six cases, for an occasional kernel carrying the *bz* mutant exhibited a small *Bz* spot. These two cases may represent extreme examples of change in rate of mutation to *Bz* associated with a change in *Ac* that does not effect its removal from the vicinity of the locus. Changes of this type are known to occur. In the remaining plant of the twenty-one, two *Ac* elements were present, one located close to *bz* and one not linked with markers in chromosome 9.

It is clear that, in at least sixteen of the twenty-four cases described, mutation to a stable recessive was associated with removal of *Ac* from the locus of bronze, and that in seven of them *Ac* was transposed to a new location.

Another type of change may accompany removal of *Ac*. It brings about substitution of the *Ds-Ac* system of control of mutation at the bronze locus for the apparently direct control of this process by *Ac*. After such a change occurs, it can be shown that a *Ds*-type element, instead of the *Ac* element, resides at the bronze locus, and that the response of this *Ds* element to *Ac* brings about the observed mutations. In the absence of *Ac*, no mutations occur. A case of this type was mentioned earlier; and two additional cases have been detected. Both of these exhibit a stable *bz* expression in the absence of *Ac*, but undergo mutations to higher alleles of *Bz* when *Ac* is present. The mode of origin of the change in type of control of the mutation process is not yet understood. It is conceivable, however, that in the original case both a *Ds* and an *Ac* element are present, located close together, and that the apparently direct *Ac* control of the mutation process is deceptive because of frequent simultaneous removals of both elements from the vicinity of the bronze locus when a mutation occurs. Another possibility is that a *Ds* element may be substituted for an *Ac* element at the time of removal of *Ac*. This substitution is readily conceivable, and could equally well account for the appearance of a *Ds*-type element at the bronze locus in the three cases mentioned above.

Control of Gene Action by a Non-transposing *Ds* Element

The effects produced by *Ds* on the action of genic materials located to either side of it, after its insertion just distal to *Sh*, were reviewed earlier (Year Books Nos. 51, 52, and 53). It induces mutations in genic substances located to its right, which affect *Sh* or both *Sh* and *Bz* simultaneously. It also induces mutation of genic substances located to its left, including the locus of *I*. Some of the mutants so produced are unstable and undergo reversions. It can be shown that the *Ds* element is also responsible for these reversions, but in none of the cases examined was *Ds* altered in location when such a reversion occurred. The *Ds* element, when inserted at some other position, is known to be readily transposable to new locations. Such transpositions are usually associated with mutation of genic substances at the locus where *Ds* has been residing. Particular attention was given, therefore, to investigating those changes in gene action induced by *Ds*, when located just to the left of *Sh*, that were readily reversible. This was done in order to determine whether the apparent fixity in position of *Ds*, after insertion just to the left of *Sh*, would be maintained in every case, as the previous evidence suggested. One such case, considered to be particularly suitable for the purpose, was examined, and the results will be reported below.

As was mentioned above, some of the *Ds*-induced modifications effected a change in expression of both *Sh* and *Bz* and resulted in the appearance of the double mutant, *sh bz*. In the case to be described, the *bz* component but not the *sh* component proved to be mutable, and mutations to higher alleles of *Bz* occurred. It was detected in the following manner. A plant homozygous for *I*, *Ds*, *Sh*, and *Bz*, and also carrying one *Ac*, was used as female parent in a cross with a plant that was homozygous for *C*, *sh*, and *bz* and had no *Ac*. An exceptional kernel appeared on the ear produced by this cross. It was *I sh* in phenotype. The plant grown from this kernel exhibited the recessive bronze phenotype, and no mutations to *Bz* were noted in the plant tissues. One ear of this plant was self-pollinated, and from the kernel types that appeared it could be concluded that one chromosome 9 carried *I*, *sh*, and *bz* and its homologue carried *C*, *sh*, and *bz*. Another ear of this plant received pollen from a plant that was homozygous for *C*, *sh*, and *bz* but also carried one *Ac*. On the ear produced by this cross it was evident that *Ds* was present in the *I sh bz*–carrying chromosome, and also that mutations to *Bz* were occurring at the *bz* locus in this chromosome, but only in those kernels that had received *Ac* from the male parent. Subsequent tests indicated that the female parent did not have *Ac*, but that the *bz* locus carried in the *I sh bz* chromosome was capable of mutating to higher alleles of *Bz* in its presence. Tests were then conducted to determine the nature of the change in the *Sh Bz* region that had originally occurred in one of the *I Ds Sh Bz*–carrying chromosomes of the parent plant, and also the conditions that governed the reversions to *Bz*. The evidence obtained from these tests is summarized below.

A *Ds*-induced mutation in an *I Ds Sh Bz*–carrying chromosome affected gene action in the segment of the chromosome that includes *Sh* and *Bz*. *Ds* was not altered in location by this event, and thus the segment was composed of the recognizable components *Ds*, *sh*, and *bz*. In all subsequent tests, this segment behaved as a unit in inheritance, for no evidence was obtained of crossing over within it. Chromosomes carrying it were normally transmitted through both pollen and egg, and individuals homozygous for it were viable and normal in appearance. In the absence of *Ac*, no modifications affecting this segment occurred; there were no dicentric-acentric-chromatid-forming events at *Ds*, and no mutations to *Bz*. In its presence, however, both occurred. The frequency of occurrence of dicentric chromatid formation was high, and that of mutation to *Bz* was low, but it was evident that both events were expressions of the presence of *Ds* in this segment and of its responses to *Ac*. The *sh* component of the segment was stable both in the presence and in the absence of *Ac*, for no mutations to *Sh* were noted. In the presence of *Ac*, a few mutations to *Bz* occurred in sporogenous cells. These were detected in individual kernels appearing on ears produced by appropriate crosses of plants that had the modified segment. Thirteen kernels representing independent occurrences of germinal mutation to *Bz* were selected from such ears, and examination of the mutant commenced with the plants derived from them. In none of the thirteen cases did the event responsible for the mutation to *Bz* affect the recessive *sh* expression, nor did it result in a change in location of *Ds*. In the absence of *Ac*, the *Bz* expression proved to be stable; but in its presence further mutations occurred, many of them again giving rise to the recessive, *bz*, expression. Some of these *bz* revertants, in turn, were unstable, and there were many mutations back to *Bz*, but only when *Ac* was present in the nucleus.

Chromosomes carrying any one of the thirteen examined *Bz* mutations were normally transmitted through pollen and egg, and plants homozygous for them were viable. The mutants, however, were not all alike. The intensity of pigmentation in some of them was less than that produced by the standard *Bz*. Also, most of the mutants did not produce the diffusible substance that allows a *Bz* phenotype to appear in a *bz* genotype, as the standard *Bz* is known to do. This substance is made evident in kernels that are sectorial for the *Bz* and *bz* genotypes. Cells in a *bz* sector that are immediately adjacent to a *Bz* sector exhibit a *Bz* phenotype because of the presence in them of a substance derived from the *Bz* cells. A few of the thirteen *Bz* mutants did produce this substance, and in all major respects these mutants were similar to the standard *Bz*. Two of the thirteen mutants received extensive study. In both, the *Ds sh Bz* segment behaved as a unit in inheritance. It could be shown, however, that the *Ds* component was located at a position distal to that of the *Bz* component, even though the two components were not separated from each other by crossing over in rather extensive tests designed to detect such separation.

This evidence accords with that obtained in all other examined cases of change in gene expression produced by *Ds* after its insertion at this one particular position in chromosome 9—just distal to *Sh*. In the presence of *Ac*, it can induce various types of change in gene action. Some of them effect a spread of mutation-type changes along the chromosome to either side of *Ds*. The extent of influence of any one event varies from an effect on *Sh* alone to an effect on all the genic substance located within the *I*-to-*Bz* interval, including these two loci. In all the many examined instances of such change in gene expression, the *Ds* element has been found to be present after the occurrence of the mutation-producing event, and its location has apparently been unaltered. In all cases of reversion in the gene expression, it has been shown that the *Ds* element was responsible and, again, that its position was not altered.

Continued Examination of the a_1^{m-1}-*Spm* System of Control of Gene Action

Since the mode of control exhibited by the a_1^{m-1}-*Spm* system was outlined in some detail in Year Book No. 54 it need not be reviewed here. During the past year, studies of this system were aimed at adding to the evidence about transposition of the *Spm* element by determining the extent to which it occurs at various stages in plant development. It was learned that, although transpositions of *Spm* may occur early in plant development, most of them occur relatively late. Progeny of plants carrying an *Spm* element at a known location in one chromosome of the complement were investigated, in order to determine the frequency of occurrence of transposition from this known location—in either chromosome 5, chromosome 6, or chromosome 9—to new locations. Disappearance of *Spm* from the known location and its appearance at a new location were detected in some individuals in each progeny.

Several different modifiers of the a_1^{m-1}-*Spm* system were also examined. One behaves as a recessive in the presence of *Spm;* in the absence of *Spm* it controls the type of a_1^{m-1} action in plant and kernel. The plant tissues develop pigment much as they do in the absence of *Spm*, as described earlier, although the rate of development is much slower. The kernels, however, are usually totally colorless; in some of them, one or several very small dots of deep pigmentation may appear. Several modifiers of this type have been observed in the a_1^{m-1} cultures, and each was found to occupy a different position in the chromosome complement. Another type of modifier that has appeared greatly enhances the frequency of occurrence of mutation at a_1^{m-1}, but only when *Spm* is also present in the complement. A third type of modifier is a system composed of two complementary elements, which are independently located in the chromosome complement. Its effects are observed in the absence of *Spm*. One of the two elements of this system is responsible for the appearance in the kernel of a regular pattern of presence and absence of a pale-colored anthocyanin pigment. When the second factor is also present, dots of deep anthocyanin pigmentation appear in the colorless areas.

It is clear that a number of different elements may be present in the nucleus, each involved in some manner in control of a_1^{m-1} action. In studies of the *Ds-Ac* system, on the other hand, such distinctive types of modifiers have not yet been recognized. Analysis of that system has therefore been relatively free from the apparent confusion that such modifiers can introduce in attempts to understand the modes of action of particular controlling systems. Nevertheless, recognition of the presence of a number of different types of element, each of which can act upon a particular known controlling element at a given gene locus, is of considerable significance in viewing the modes of operation of controlling elements and their integrative action in the nucleus. Just such complexity of relations is to be expected if controlling elements play a significant part in modifying gene action within the nucleus. If such modifiers were not found, each system would seem to behave as an isolated unit, and its relation to integrative mechanisms within the nucleus would not be apparent. Various levels of integration of controlling systems are to be expected. The modifiers described here may represent a second level of integration.

Changes in Chromosome Organization and Gene Expression Produced by a Structurally Modified Chromosome 9

A structural modification affecting the organization of chromosome 9, which is responsible for inducing other structural alterations both in chromosome 9 and in other chromosomes of the complement, has been examined. In this case, the substance of a normal chromosome 9 is divided into two chromosomes of quite different lengths. The smaller chromosome is composed of the distal third of the short arm of the normal chromosome 9, and carries the loci of *Yg* and *C*. At its proximal end is a centromere, from which extends a short piece of deeply staining chromatin of unknown origin; but the extension is often lost from the chromosome, leaving it with a terminal centromere. This short member of the structural modification will be referred to as the fragment chromosome. The longer member is composed of the proximal two-thirds of the short arm of chromosome 9 and all of its long arm. The locus of *Sh* is close to the end of the short arm of this chromosome, and the loci of *Bz* and *Wx* follow in the normal order. This member will be referred to as the deficient chromosome.

Cytological examination of various meiotic stages was made in plants that were either heterozygous or homozygous for this structural modification. In the heterozygote, the deficient chromosome was always synapsed with homologous parts of the normal chromosome 9 throughout all of its long arm, and usually also throughout most of its short arm. At the pachytene stage, the fragment chromosome in most cells was found to be synapsed with homologous parts of the normal chromosome 9 throughout much of its length. In some cells, however, it was completely unassociated, lying free in the nucleus. Particular attention was given to those cells in which synapsis of both components of the modified chromosome 9 with homologous parts of the normal chromosome was fully expressed. They furnished no evidence of either duplication or deficiency of parts of chromosome 9 within either of the two components of the structurally modified chromosome; the fragment chromosome and the deficient chromosome appear to represent a complete chromosome 9. It should be emphasized, however, that, in many of the cells in which the fragment chromosome was completely synapsed with its homologous part in the normal chromosome, the centromere of the fragment was closely appressed to the adjacent region in the normal chromosome, where *Sh* and *Bz* are located. When this association was observed, the *Sh*-and-*Bz*–carrying region in the deficient chromosome, located near the end of its short arm, was not synapsed with the homologous region in the normal chromosome.

The deficient chromosome is transmitted through the pollen grain only when the fragment chromosome is also present in the tube nucleus. Through the female gametophyte, however, it is transmitted without the accompanying fragment. Thus, plants may be obtained that have a normal chromosome 9 and a deficient chromosome 9 but no fragment. When the deficient chromosome carries *Sh, Bz,* and *Wx* and the normal chromosome carries the recessive alleles, crossing over within the *Sh*-to-*Bz* and *Bz*-to-*Wx* regions may be determined readily by using plants of this constitution as pollen parents in crosses to plants that are homozygous for *sh, bz,* and *wx*. Among a total of 16,514 kernels obtained from crosses of this type, only 0.4 per cent carried a chromosome that could have undergone crossing over within the *Sh*-to-*Bz* region. This figure represents a marked reduction from the standard value of 1.5 to 2 per cent. Within the *Bz*-to-*Wx* region, however, the standard frequency of crossing over was exhibited, amounting to 18.6 per cent. All but 5 of the kernels from these crosses were *sh* in phenotype. The 5 exceptional kernels were all *Sh Bz Wx* in phenotype.

A number of crosses similar to those just described were conducted with plants that had a fragment chromosome in addition. In these plants, the deficient chromosome carried *Sh* and *Wx* and the normal chromosome carried the recessive alleles, *sh* and *wx*. They were crossed to plants that were homozygous for *sh* and *wx*. Because of nonregulated disjunctions of the fragment chromosome at the first meiotic anaphase, and also because of frequent noninclusion of the fragment in either a telophase I or a telophase II nucleus, the number of pollen grains that carried both the deficient chromosome and the fragment was considerably lower than the number that carried a normal chromosome, with or without the fragment. Because the deficient chromosome carrying *Sh* is transmitted through the pollen only when the fragment is also present, the number of *Sh* kernels on ears produced by a test cross of this kind should be considerably smaller than the number of *sh* kernels. Also, because a crossover in the distal third of the short arm—between the normal chromosome and the fragment—interferes with a crossover in the region proximal to it, and because such a crossover leads to chiasmal association of the fragment and the normal chromosome at metaphase I and consequently to disjunction of the fragment at anaphase I and its inclusion in two of the four nuclei at telophase II, the percentage of pollen grains carrying the fragment and the deficient chromosome with a crossover in the *Sh*-to-*Wx* region (the *Sh wx* class) should be smaller than the percentage of pollen grains carrying a normal chromosome with the reciprocal crossover (the *sh Wx* class). In the light of these statements, the ratios of kernel types appearing on the ears produced by the test cross may be readily interpreted. They were as follows: 4072 *Sh Wx*:524 *Sh wx*: 4319 *sh Wx*:20,081 *sh wx*.

A test of the above-described types was conducted with five plants that had the markers *c, sh, Bz,* and *wx* in the normal chromosome 9, *c* in the fragment chromosome, and *Sh, Bz,* and *Wx* in the deficient chromosome. When they were used as pollen parents in crosses with plants homozygous for *C, sh, bz,* and *wx,* an unexpected class of kernels appeared. These kernels exhibited the recessive *bz* phenotype, and all of them were *sh*. They appeared in constant proportions, as shown in section B of table 4. Another plant, of similar constitution with respect to markers in the normal chromosome 9 and the deficient chromosome but having no fragment present, was also crossed to plants homozygous for *C, sh, bz,* and *wx;* and from this cross no *bz* kernels resulted, as shown in section A of the table. Apparently, the fragment is in some way responsible for the appearance of kernels exhibiting the *bz* phenotype. This was also suggested by a test conducted with plants that had *c, sh, Bz,* and *wx* in the normal chromosome 9, *C* in the fragment, and *Sh, Bz,* and *Wx* in the deficient chromosome. They were used as female parents in crosses with plants that were homozygous for *c, sh, bz,* and *wx*. Among the 506 kernels on the ears produced, 377 were *c* (53 *Sh Wx*:12 *Sh wx*:18 *sh Wx*:294 *sh wx*) and 129 were *C*. Among the *C* kernels, the expected phenotypes appeared (84 *Sh Bz Wx*:4 *Sh Bz wx*:1 *sh Bz Wx*:35 *sh Bz wx*), but in addition there were 5 exceptional kernels, *C sh bz wx* in phenotype. The appearance of kernels exhibiting the *bz* phenotype might be explained on the assumption that the fragment chromosome carries *bz* and that crossing over occurs between this marker and the centromere of the fragment. This explanation would require that the fragment carry a duplicated piece of chromatin, for it is known that the loci of *Sh* and *Bz* are in the deficient chromosome. But, as was stated earlier, no evidence of such a duplication was seen. Moreover, if this explanation were correct, a crossover in this region would be expected to interfere with one to the right of it—between *Bz* and *Wx*. No such interference was expressed, as the data in table 4 indicate.

In order to initiate investigation of the nature of the change in chromosome 9 that was responsible for the altered *Bz* expression, the 41 *bz* kernels entered in table 4, as well as 4 of the 5 *bz* kernels produced by the second cross, were sown and plants were obtained from them. All forty-five plants exhibited the *bz* phenotype. Sporocytes were collected from all the plants, and from them the chromosome 9 constitution in each plant could be determined. Forty-four of the forty-five plants had two normal-appearing chromosomes 9; in seven of the forty-four, the fragment chromosome was also present. No modification in the *bz*-carrying region of one chromosome 9 was obvious in any of these plants. In the remaining plant of the forty-five, however, a modification was clearly observed. This plant had one normal chromosome 9, a fragment chromosome, and a deficient chromosome; but a small piece of chromatin was missing from the end of the short arm of the deficient chromosome. The loci of *Sh* and *Bz* had been deleted, and because of this the *sh bz* phenotype had appeared. It is clear, nevertheless, that most changes from *Bz* to *bz* are not associated with any gross change in chromo-

TABLE 4

DATA SHOWING THE FREQUENCY OF APPEARANCE OF THE *bz* PHENOTYPE IN PROGENY OF PLANTS CARRYING THE FRAGMENT CHROMOSOME (*B*, BELOW), AND THE ABSENCE OF THIS PHENOTYPE IN PROGENY OF PLANTS THAT DID NOT HAVE THE FRAGMENT CHROMOSOME (*A*, BELOW)

A. ♀ *C sh bz wx/C sh bz wx* × ♂ $\dfrac{sh\ Bz\ wx;\ \text{normal chromosome 9}}{Sh\ Bz\ Wx;\ \text{deficient chromosome 9}}$

B. ♀ *C sh bz wx/C sh bz wx* × ♂ Same as A, but fragment also present

Phenotype of kernel	A	B (plants 1 to 5)					
		1	2	3	4	5	Totals for B
Sh Bz Wx	0	81	141	147	169	249	787
Sh Bz wx	0	4	15	12	27	33	91
sh Bz Wx	71	65	81	95	147	154	542 *
sh Bz wx	367	357	335	454	480	602	2229
sh bz Wx	0	2	1	1	3	2	9 †
sh bz wx	0	5	3	6	6	12	32
Totals	438	514	576	716	832	1052	3690
% *bz* among *sh* class	0	1.6	0.95	1.2	1.4	1.8	1.4

* Equals 19.5 per cent of *sh Bz* class.
† Equals 21.9 per cent of *sh bz* class.

some composition. It is possible that during the meiotic process, when the fragment is synapsed with the normal chromosome, the fragment becomes joined to the normal chromosome, at the region of its centromere, by a mechanism that simulates the crossover process. This region of joining would be situated at the part of the normal chromosome containing the loci of *Sh* and *Bz*. It is known that the fragment may attach itself at its centromere region to ends of chromosomes, with associated loss of its centromere activity. In the homozygote, the fragment can thus join with the end of the deficient chromosome, and so re-establish a structurally normal chromosome 9. Twelve independent cases of this event have been examined. They were detected because of change in expression of *Sh,* or of both *Sh* and *Bz,* in the deficient chromosome to produce either *sh* or the double mutant *sh bz*. In these twelve cases there was no evidence of deficiency in the *sh*- or the *sh bz*-carrying region of the reconstructed chromosome 9, or of any centromere activity at the position of union. The reconstructed chromosome behaved in mitosis like a normal chromosome 9. Even though the evidence obtained so far is not extensive, it is sufficient to indicate that the fragment chromosome is responsible for initiating the described modifications in gene expression in the *Sh*-and-*Bz*-carrying region of chromosome 9.

The fragment chromosome initiates types of modification other than those described above. The events responsible for them may occur in either somatic or sporogenous cells. Cytological examinations were made of sixty-two plants derived from kernels whose phenotypes had suggested alteration in constitution of the fragment chromosome itself. From these observations it was possible not only to learn what types of change in constitution of the fragment may occur but also to discover some of the alterations the fragment can induce in the constitution of other chromosomes of the complement. The modifications observed include nondisjunction of the fragment; "misdivision" of its centromere, resulting in isochromosome formation; ring-chromosome formation; attachment of the centromere of the fragment to the centromere of another chromosome of the complement, effecting union of the fragment with one arm of the other chromosome; and, as mentioned above, attachment of the centromere of the fragment to the end of another chromosome, effecting union of the fragment with this chromosome. Other, more complex types of interchromosomal modifications involving the fragment were also noted. The times and frequencies of occurrence of these events appear to be under genetic control. That tissues of plants carrying the fragment often exhibit a rather precise pattern of occurrence of such events indicates a regulated frequency.

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